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A constrained maximum likelihood approach to evaluate the impact of dose metric on cancer risk assessment: Application to β-chloroprene

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ABSTRACT

β-Chloroprene (2-chloro-1,3-butadiene, CD) is used in the manufacture of polychloroprene rubber. Chronic inhalation studies have demonstrated that CD is carcinogenic in B6C3F1 mice and Fischer 344 rats. However, epidemiological studies do not provide compelling evidence for an increased risk of mortality from total cancers of the lung. Differences between the responses observed in animals and humans may be related to differences in toxicokinetics, the metabolism and detoxification of potentially active metabolites, as well as species differences in sensitivity. The purpose of this study was to develop and apply a novel method that combines the results from available physiologically based kinetic (PBK) models for chloroprene with a statistical maximum likelihood approach to test commonality of low-dose risk across species. This method allows for the combined evaluation of human and animal cancer study results to evaluate the difference between predicted risks using both external and internal dose metrics. The method applied to mouse and human CD data supports the hypothesis that a PBK-based metric reconciles the differences in mouse and human low-dose risk estimates and further suggests that, after PBK metric exposure adjustment, humans are equally or less sensitive than mice to low levels of CD exposure.

1. Introduction

β-Chloroprene (CD, CAS# 126-99-8, 2-chloro-1,3-butadiene) is a compound used in the manufacture of polychloroprene rubber. Chronic inhalation studies in animals have demonstrated that CD is carcinogenic in B6C3F1 mice and Fischer 344 rats in multiple target organs (lung, liver, circulatory systems, forestomach, Harderian gland, kidney, mammary gland, mesentery, oral cavity, skin, and thyroid gland) (Melnick et al., 1999; National Toxicology Program, 1998). In addition, respiratory and liver cancers have been associated with CD exposure in several epidemiological studies (Acquavella and Leonard, 2001); however, interpretation of these findings has been difficult due to methodological limitations, including the inability to assign quantitative values for CD exposures, the small number of observed outcomes, and the small sample sizes for occupational studies (Marsh et al., 2007a). This makes the comparison of estimates of risk based on animal versus human results difficult.

While epidemiological studies are available for chloroprene, due to the uncertainties in the epidemiological studies the most recent quantitative risk assessment conducted by the USEPA (2010) used only animal data. The resulting cancer unit risk is driven by the most sensitive endpoint in animals, the incidence of lung tumors in female mice. Integration of the epidemiological studies does not provide compelling evidence for an increased risk of mortality from total cancers of the lung following inhalation exposure to chloroprene (Marsh et al., 2007a,b).

Previous studies have examined differences in toxicokinetics between animals and humans to determine if this is potentially

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the contributing factor to the differences in response between animals and humans. The initial step in metabolism is oxidation forming a stable epoxide, (1-chloroethenyl) oxirane, a genotoxicant that might be involved in the observed carcinogenicity in animals (Himmelstein et al., 2004b). Differences between the responses observed in animals and humans may be related to differences in toxicokinetics, to the metabolism and detoxification of potentially active metabolites (Himmelstein et al., 2004a,b), as well as to differences in species sensitivity. Specifically, Himmelstein et al. (2004a) found that the oxidation (Vmax/Km) of CD in liver was slightly faster in rats and mice than in humans and hamsters, and in lung microsomes was much greater for mice compared to other species. In addition, hydrolysis (Vmax/Km) of (1-chloroethenyl) oxirane, in liver and lung microsomes, was faster for humans and hamsters than for rats and mice.

In current risk assessments for chloroprene (USEPA, 2010), external exposure estimates are relied upon, which does not consider species differences in toxicokinetics. These differences may be critical in characterizing the potential risk of cancer following exposure to chloroprene, especially if the generation of a metabolite is related to the potential for cancer risk. The availability of physiologically based kinetic (PBK) models for both mice and humans (Yang et al., 2012) provides a unique opportunity for comparison of animal and human risk estimates based on external and internal exposure metrics. The PBK model for chloroprene incorporates the available data regarding species differences in metabolism of chloroprene. Application of the model allows for species-specific estimation of internal exposure metric, specifically the amount of chloroprene metabolized per gram of lung tissue. Risk estimates can then be compared across species based on this equivalent internal exposure metrics rather than external air concentrations.

The purpose of this study was to develop and apply a novel method that combines the results from available PBK models for chloroprene with a statistical maximum likelihood approach to test commonality of low-dose risk across species. This method allows for the combination of human and animal cancer study results to evaluate the difference between risk estimates obtained using both external and internal dose metrics.

The maximum likelihood approach applied allows for the evaluation of the ability of traditional dose–response models, such as the Multistage model, to describe the response pattern under the constraint of equal risk at a dose of interest (either internal or external), specifically a possible point of departure (POD). The results provide a demonstration of which dose metric provides statistically equivalent human- and animal-based risk estimates. Additional analyses were also conducted to investigate the impact of uncertainty in the estimated exposure levels for the human occupational study and to address the question of potential cross-species pharmacodynamic differences.

2. Material and methods

The method described here requires both animal data (a well-conducted two-year bioassay) and epidemiological data sufficient to allow dose-response analysis. Rather than modeling them separately, the approach adopted is to jointly model the selected studies to determine if, and under what circumstances, risk estimates of interest can be determined to be consistent across species. Jointly modeling the data requires software that allows for constrained maximization of the combined likelihood of the animal and human dose–response relationships with testing of hypotheses based on the comparison of the constrained maximum likelihood to the unconstrained (separate) likelihoods for the two species. Fig. 1 depicts the overall procedure.

2.1. Animal data

A two-year inhalation study of CD was conducted in F344/N rats and B6C3F$_1$ mice (National Toxicology Program, 1998). This is the bioassay relied upon by the Environmental Protection Agency (EPA) in the recent CD Integrated Risk Information System (IRIS) assessment (USEPA, 2010). Groups of 50 males and 50 females were exposed by inhalation for 6 h per day 5 days per week for 2 years to 0, 12.8, 32 or 80 ppm of CD. The National Toxicology Program (NTP) (1998) concluded that there was clear evidence of carcinogenicity in both the rats and mice following inhalation exposure to CD. In the F344/N rats, this conclusion was based on the increased incidences of neoplasms of the thyroid gland and kidney in males and females, increased incidences of neoplasms in the lung in males only and in the oral cavity and mammary gland in females only. In the B6C3F$_1$ mice, the conclusion of clear evidence of carcinogenicity was based on the increased incidence of neoplasms in the lung, circulatory system, forestomach and Harderian gland in both sexes, in the kidney for males only and the mammary gland, liver and skin for females only (see Table 5-4 in USEPA, 2010).

Based on the NTP (1998) results, USEPA (2010) concluded that that mouse is the most sensitive species, due to the increased tumor incidence and multisite distribution in the mouse relative to the rat. The EPA calculated a composite unit risk from all the female mice cancer endpoints listed above (9.8×10^{-1} per ppm; 2.7×10^{-4} per $\mu g/m^3$), and the unit risk estimated from the combined incidence of lung adenomas or carcinomas in the female mice produced the highest site-specific unit risk (6.4×10^{-1} per ppm; 1.8×10^{-4} per $\mu g/m^3$). As it was the most sensitive of the site-specific endpoints, combined lung adenomas and carcinomas is the endpoint considered in the current analysis. Analyses of rat responses, and perhaps additional mouse responses, may follow, given the success of this investigation.

2.2. Human data

Marsh et al. (2007a,b) conducted a historical cohort study to investigate the mortality of industrial workers potentially exposed to CD and other substances (including a potential confounding co-exposure to vinyl chloride). This study represents one of the most recent epidemiological studies and the design attempted to address the problems identified with earlier studies by conducting a detailed exposure assessment for both chloroprene and vinyl chloride monomer. The emphasis of the study was on cancer mortality, including respiratory system cancer. Four different CD production sites (i.e., Louisville, KY; Pontchartrain, LA; Maydown, Northern Ireland; and Grenoble, France) were included in the Marsh et al. study. The Louisville cohort examined by Marsh et al. (2007a,b) had the greatest number of exposed individuals, the greatest number of person-years of follow-up, and the greatest average exposure level (both in terms of the intensity level, ppm, and in terms of cumulative exposure, ppm-years). The greater exposure levels, combined with the greatest number of exposed individuals, increase the probability of detecting any carcinogenic effect following exposure to CD. Respiratory system cancer mortality from the Louisville cohort was used in this analysis as those data came from the best epidemiological dataset available (in terms of adequacy of size and suitability for dose–response analysis) that measured an endpoint that was comparable to the most sensitive endpoint in mice. The other cohorts may be subject to future analyses; inclusion of additional cohorts may increase the power of the epidemiological modeling.

For the Louisville cohort, approximate quartiles of the data were determined by Marsh et al. (2007b) based on the distribution of death from all cancers, and these quartiles were used to define

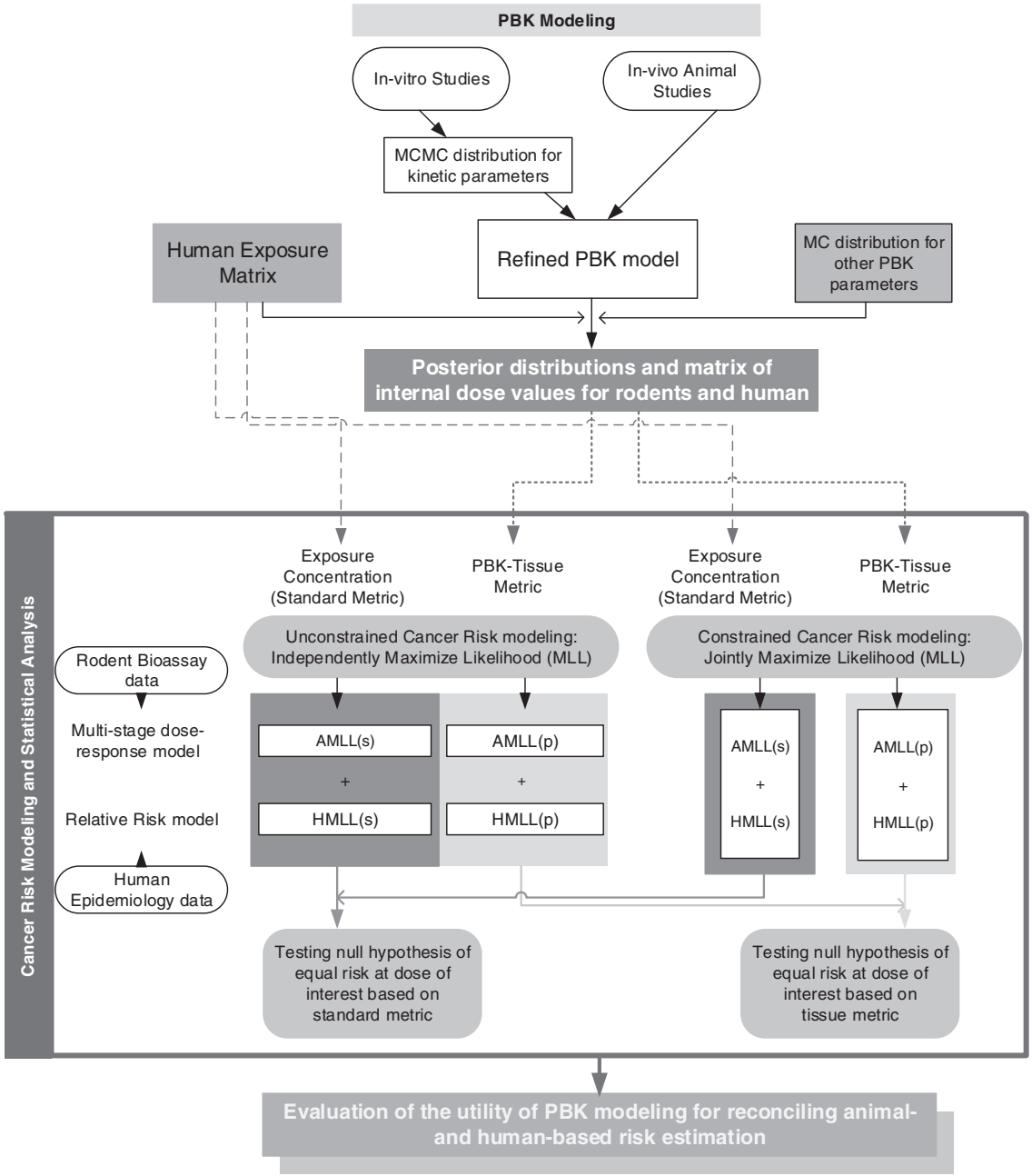

Fig. 1. Overview of physiological based kinetic modeling probabilistic dose response modeling.

the subgroups for all other cancer types, including the respiratory cancer used in this analysis. The exposure reconstruction detailed in Esmen et al. (2007b) was used, in combination with the Occupational Cohort Mortality Analysis Program (OCMAP) (described in detail in Marsh et al., 1998) to determine the quartile-specific and overall average cumulative exposure.

2.3. Estimation of exposure/dose

In the evaluation of the animal data, external air concentrations used in the exposure-response modeling were the administered air concentrations in the NTP (1998) study in ppm adjusted to an equivalent continuous exposure, adjusting for hours per day (6/24) and days per week (5/7) (Table 1). Similarly, the human cumulative doses were adjusted from occupational to continuous exposure by adjusting for the number of work weeks per year (50/52), for work days per week (5/7) and for percentage of total daily inhalation that occurs during work hours (10/20) (USEPA, 2009). Adjusted values are shown in Table 2.

Based on the range of reported exposures for each quartile, the midpoints of cumulative exposure for the first three exposure groups were used (assumed to characterize the respective group average exposure for dose–response modeling). However, because the high exposure group was characterized as 164.053+ ppm-years with no highest exposure value, an approach was needed to characterize the average exposure for this group (Table 2). The average exposure used for the highest group was calculated based on the midpoint values for exposure groups 1 through 3, the overall average cumulative exposure computed by OCMAP, and the number of person-years apportioned to each group, shown here:

Table 1
Animal data modeled via the multistage model.

Dose group	Continuous exposure equivalent (ppm)	PBK metric (μmole/g-lung/day)	Group size	Number of animals with respiratory system cancer
1	0	0	50	4
2	2.3	0.705	49	28
3	5.7	1.12	50	34
4	14.3	1.47	50	42

Table 2
Human data modeled via a linear relative risk model.

Cumulative exposure group	Published cumulative exposure ranges (ppm-years)	Average cumulative exposure (ppm-years)	Assumed adjusted average cumulative exposure (ppm-years)	PBK metric (μmole of metabolite/g lung/day-years)	Person years of observation	Deaths from respiratory system cancer	SMR	Computed expected
1	<4.747	2.37	0.814	0.0083	68918	62	0.71	87.32
2	4.747–55.918	30.3	10.4	0.107	56737	67	0.71	94.37
3	55.918–164.052	110	37.8	0.387	39840	77	0.92	83.70
4	164.053+	297[a]	102	1.05	32424	60	0.65	92.31

[a] Calculated using text Eq. (1).

$$\text{ppm-years}(\text{avg},\text{total}) = \left[\sum \text{ppm-years}(\text{avg},i) * \text{PY}(i)\right]/\text{PY}(\text{total}) \quad (1)$$

where ppm-years(avg, total) is the average cumulative exposure for the entire cohort (80.35 ppm-years), ppm-years(avg, i) is the assumed average cumulative exposure for groups 1-3 or the unknown X ppm-years for group 4; PY(total) is the total number of person years of follow-up for the cohort (197919); and PY(i) is the person years of follow-up for group i (68918, 56737, 39840, and 32424 years for groups 1 through 4, respectively). The values for the ppm-year ranges and person years of follow-up (see also Table 2) are from Marsh et al. (2007b). The only unknown in the equation above, X, is for the ppm-years for group 4. Solving for X gives an estimate of the cumulative exposure for group 4 of 297 ppm-years.[1]

An internal dose metric (PBK metric) was estimated for both the animal and human datasets using the PBK model by Yang et al. (2012). Following Markov Chain Monte Carlo (MCMC) analyses, Yang et al. derived a set of posterior distributions for each of the kinetic parameters in both the mouse and the human PBK models. The mean from each distribution (i.e., one for each kinetic parameter) as well as the standard physiological and partition coefficient values (Yang et al., 2012) for each species were used in the corresponding PBK model to derive the internal dose metric of μmoles of metabolized CD/g lung/day for each exposure group in both the mouse experimental study and the human occupational study. Such a metric reflects the estimated metabolism of CD to reactive metabolites, including (1-chloroethenyl) oxirane, which are the proposed carcinogenic moieties (Yang et al., 2012). Since metabolism of CD is different between mice and humans, the use of PBK model estimates of internal dose, as a measure of exposure, provides a method to account for these species-specific differences.

For both the mouse and the human, the models were run for a week-long exposure (5 days per week). It was observed that after the 2 (weekend) days of non-exposure, chloroprene was cleared from the body for both species. Thus, a single week of modeling the experimental exposures or occupational exposures was sufficient to calculate the lifetime daily average.

[1] This approach used to determine the average concentration for the highest exposure group was deemed preferable to using a midpoint between 164 ppm-years and 1351.5 ppm-years, the reported maximum seen in the cohort. The dose for the highest group would have been larger (758 ppm-years) and would not have maintained the reported average ppm-year value for the entire cohort. Rather than relying upon a midpoint of the range of exposure, the consideration of average values for grouped exposure summaries in the current approach reflects all of the available information regarding cohort exposure.

2.4. Calculation of animal-based risks

For the current assessment, the Multistage model provided in the USEPA Benchmark Dose Software (BMDS) program (USEPA, 2012) was fit to the female mice lung adenoma or carcinoma incidence data using the continuous exposure equivalent in ppm (adjusted from 6 h per day 5 days per week to continuous). In addition, the model was also fit to the data using the internal PBK metric of μmole CD metabolized/g of lung/day obtained from simulations of the Yang et al. (2012) PBK model (Table 1).

The multistage model has the mathematical form:

$$P(d) = 1 - e^{(-q_0 - q_1 \times d - \ldots q_k \times d^k)} \quad (2)$$

where d is the average lifetime daily dose, $P(d)$ is the lifetime probability of tumor from the dose level d, and $q_0,\ldots,q_k$ are nonnegative parameters estimated by fitting the model to experimental animal data. The multistage modeling performed in this analysis assumed $k = 2$, i.e., it used a two-stage model.

The multistage model is a flexible statistical model that can describe both linear and non-linear dose-response patterns. It has been used as the standard for cancer risk analysis, and for many years the default dose-response model for federal and state regulatory agencies in the United States for calculating quantitative estimates of low-dose carcinogenic risks from animal data (USEPA, 1986, 2005).

The choice of a low-dose extrapolation method used by the EPA, in particular, in dose–response assessments should be informed by the available information on the mode of action of cancer, as well as other relevant biological information, and not solely on goodness-of-fit to the observed tumor data (USEPA, 1992). However, when data are limited or when uncertainty exists regarding the mode of action, models which incorporate low-dose linearity are the default approach. EPA usually employs the linearized multistage procedure in the absence of adequate information to the contrary; many of the available IRIS values are based on the results from this model. In that capacity, it is regularly used on data sets with only a few data points as is common for animal studies.

Using the external and internal dose metrics for CD, a single maximized log-likelihood was determined for each: the

unconstrained animal maximum log-likelihood for the standard (or external) metric (AMLLs) and the unconstrained maximized log-likelihood for the internal metric (AMLLp) (Fig. 1). Each of the AMLLx values represents the usual data-specific measure of the fit of the model to the animal bioassay results and is the maximum value of that log-likelihood with no other constraints.

2.5. Calculation of epidemiology-based risks

A linear relative risk model was fit to the summarized data from the Louisville cohort used in this analysis (Table 2).[2] The assumed average cumulative exposure, the observed deaths from respiratory system cancer, and the expected deaths from respiratory cancer were used in a linear model to estimate the relative risk:

$$\text{Relative Risk} = \text{Observed}/\text{Expected} = \alpha * (1 + \beta \text{d}) \tag{3}$$

where d is a measure of cumulative exposure and α and β are parameters to be estimated. "Expected" was computed as the observed number of cases ("Observed") divided by the Standardized Mortality Ratio (SMR). Fitting to the human epidemiological data (Table 2) was accomplished via Poisson maximum likelihood techniques (Frome, 1983). The log-likelihood for the assumed Poisson distribution in a group having cumulative exposure d is expressed as:

$$\text{LL} = -\text{Expected} * \alpha * (1 + \beta \text{d}) + \text{Observed} * \ln(\text{Expected} * \alpha * (1 + \beta \text{d})). \tag{4}$$

This log-likelihood ignores terms that are constant for the data set (i.e., do not depend on the values of the parameters). The maximum total log-likelihood (summed over each exposure group) was obtained and retained for future computations, as HMLLs or HMLLp, corresponding to the unconstrained human log-likelihood for the standard and PBK metrics, respectively.

2.6. Human–animal comparison of chloroprene risk estimates

The current method was developed to test the null hypotheses that certain dose metrics would provide comparable risk estimates across species, specifically mice and humans. The approach was designed to determine if one or more of the selected dose metrics was consistent with the hypothesis that there was a common risk level (across species) associated with a dose or exposure pattern of interest. The alternative hypothesis, for a given dose metric, was that the risk at the dose of interest was not the same across species.

Preliminary analyses had suggested that the benchmark dose at the extra risk level of 0.10 (BMD10) from the multistage dose–response model was just slightly less than 1 ppm, so this air concentration was selected as a reasonable concentration for comparison of risk estimates across species. For the PBK metric comparison, a value of 0.00352 μmole of CD metabolized/g-lung/day was selected as the internal dose metric of interest as that was the value estimated with model simulations conducted at either 1 ppm via an occupational exposure scenario or with the adjusted continuous exposure equivalent of 0.33 ppm.

For the ppm metric (the standard metric), a single maximized log-likelihood was determined, the unconstrained animal maximum log-likelihood for the standard metric (AMLLs) (Fig. 3). For the PBK metric, the maximum log-likelihood (AMLLp) was computed in exactly the same manner, but using the PBK metric values as the dose inputs (Table 1). Correspondingly, calculation of human relative risks was conducted by fitting the relative risk model (Eq. (3)) to the epidemiology data to define the dose–response relationship using both the standard metric (with maximum likelihood HMLLs) and the PBK metric (yielding HMLLp). Using the animal and human log-likelihood estimates, unconstrained joint log-likelihoods of observing both the animal bioassay results and the epidemiological results were computed. The joint log-likelihoods were defined as "Unconstrained" meaning that the human and animal results were computed independently of one another. The computed unconstrained joint log-likelihoods (UMLLs and UMLLp) were determined based on the animal and human maximized log-likelihoods:

$$\text{UMLLs} = \text{AMLLs} + \text{HMLLs} \tag{5}$$

$$\text{UMLLp} = \text{AMLLp} + \text{HMLLp} \tag{6}$$

i.e., the metric-specific summation of the corresponding animal and human maximized log-likelihoods.

Constrained log-likelihoods were also calculated based on the null hypothesis that the animal bioassay data and the epidemiology data would provide the same estimate of risk at the dose of interest (1 ppm or 0.00352 μmole of CD metabolized/g-lung/day, depending on the metric under consideration). A joint log-likelihood for the combined human and animal results was calculated, under the assumption of equal risks at the dose of interest. If this constrained joint log-likelihood was sufficiently close to (by a formal statistical test) the unconstrained joint log-likelihood, then the null hypothesis of equal risks at those dose values was accepted.

The constrained maximum likelihood of interest was computed by examining values of β in the relative risk model (Eq. (3)), within a range of β values extending from 0 to an upper limit sufficient (by visual inspection) to guarantee that the maximum joint constrained log-likelihood was attained. For a selected value of β, the value of α in Eq. (3) was derived that maximized the human log-likelihood. In addition, for any selected value of β, a lifetime extra risk was calculated using the life table method used by EPA and others (Federal Register, 2004; USEPA, 2002, 2011) (Appendix A). The reference population for the life table calculations was the entire US population with rates from 2008 for all causes and respiratory system cancers (CDC, 2011). Risk was computed up through age 85. The lifetime human extra risk (HER) for a selected constant exposure level (dose-of-interest, or DOI) was computed using the life table approach with the various estimates of β; it was referred to as the HER(DOI).

Given the HER(DOI) value defined above, the multistage model was fit to the animal data with an added constraint, i.e., that the animal extra risk at the DOI, AER(DOI), equals the HER(DOI). The source code for the BMDS multistage model was modified (code supplied by the authors on request) to allow for such constrained optimization; it is not possible to do it with the BMDS models as they are distributed. The modification automates the following calculations. If AER(DOI) is set equal to HER(DOI), then the multistage fit to the animal data can be maximized under that constraint:

$$\begin{aligned} \text{HER}(\text{DOI}) = \text{AER}(\text{DOI}) &= [P(\text{DOI}) - P(0)]/[1 - P(0)] \\ &= 1 - e^{(-q_1 \text{DOI} - q_2 \text{DOI}^2)} \end{aligned} \tag{7}$$

where the second equality follows from the form of the multistage model equation (Eq. (2)). Solving for q_1, results in the following equation.

$$q_1 = [-\ln(1 - \text{AER}(\text{DOI})) - q_2 \text{DOI}^2]/\text{DOI} \tag{8}$$

Consequently, when AER(DOI) is fixed at a value, HER(DOI), the optimization for estimating the maximum (constrained) likelihood

[2] Even though the individual data for this cohort were available to the authors, we have used the summary data in order to demonstrate how this approach can be implemented with data that are commonly available when using epidemiological study reports for risk assessment. If we had used the individual data, we could, for example, have used a Cox proportional hazards model to better control for other variables, like age.

from the multistage model can be accomplished by varying q_0 and q_2. (i.e., all the parameters other than q_1) and then computing q_1 as shown. For the current investigation, a 2nd degree multistage model was the highest polynomial degree needed. The same assumptions would apply for a polynomial degree greater than 2.

The two log-likelihood components, human and mouse, were then summed:

$$\mathrm{CMLLx}(\beta) = \mathrm{HMLLx}(\beta) + \mathrm{AMLLx}(\beta), \tag{9}$$

indicating the dependence on the choice of β. The value of "x" in Eq. (9) was either s (for the standard, ppm metric) or p (for the PBK metric), just as for the unconstrained likelihood calculations. The full range of allowable β values was examined to determine a maximum for CMLLx(β); that maximum was the maximum constrained log-likelihood, CMLLx.

A likelihood ratio test was used to test the null hypothesis that the constraint of equal risks at DOI was true. The test statistics were:

$$2 * (\mathrm{UMLLx} - \mathrm{CMLLx}) \tag{10}$$

(twice the differences in the log-likelihoods, $x = s$ or p). There is one degree of freedom associated with the chi-squared distribution that approximates the distribution of those test statistics (Eq. (8) demonstrates there is one less parameter to be estimated, i.e., q_1, when the constraint of HER(DOI) = AER(DOI) is in effect, that is, when the null hypothesis is true). Larger differences in the maximized likelihoods yield larger values of the test statistic and therefore smaller p-values (i.e., probabilities of being in the tail of the chi-squared distribution to the right of the test statistic value). Small p-values (less than 0.05) were indicative of the null hypothesis being false.

2.7. Uncertainty analyses

An uncertainty analysis was conducted to evaluate the potential impact of the assignment of CD exposure concentrations (ppm) to the workers in the Louisville cohort. Esmen et al. (2007a) assigned nominal exposure levels to the members of the Louisville cohort, depending upon job class and calendar year. The uncertainty in the nominal levels was considered using "subtitles" for jobs within job class, the type of rotation among workers within those subtitles, and the deciles of the varying exposure levels associated with those subtitles. A Monte Carlo analysis was conducted, generating 3000 simulated human data sets, to evaluate the impact of exposure uncertainty. Each simulated human data set assigned different ppm exposure levels to each worker's work history, consistent with exposure uncertainty distributions defined in the Supplemental material; a detailed description of the approach used in the Monte Carlo for the assigning of exposures concentrations to the workers is provided in that Supplemental material.

Given the rules specified in the Supplemental material, 1500 alternative (simulated) exposure histories for the cohort members were generated and run through the OCMAP program (Marsh et al., 1998). The output of each of those runs was a set of dose–response data analogous to those shown in Table 2. The cut points for defining the exposure groups were the same as used in the original analysis (Marsh et al., 2007b) (second column of Table 2).

When considering the PBK metric for humans, the above procedure was used to generate another set of 1500 simulated data sets, but an additional step was included to represent the uncertainty between the ppm exposure level and the PBK dose metric value. That additional step utilized the posterior distributions of the PBK model parameters derived by Yang et al. (2012). Following the assignment of each ppm exposure level as described in the Supplemental material, a PBK metric value was generated by sampling from a lognormal distribution with (natural scale) mean and coefficient of variation equal to,

Table 3
Heuristic for comparing models via Bayesian Information Criteria (BIC) values.

ΔBIC[a]	Strength of evidence
<−10	Very strong evidence *for* model *i*
−10 to −6	Strong evidence *for* model *i*
−6 to −2	Positive evidence *for* model *i*
−2 to 2	Not much evidence either way
2 to 6	Positive evidence *against* model *i*
6 to 10	Strong evidence *against* model *i*
>10	Very strong evidence *against* model *i*

[a] ΔBIC = BIC(i) − BIC(j), where BIC(k) is the BIC associated with model k. Based on the categorization shown in Kass and Raftery (1995).

$$\begin{aligned} \mu &= 0.00373^{*}\mathrm{ppm} \\ \mathrm{CV} &= 0.74, \end{aligned} \tag{11}$$

respectively. Those values for μ and coefficient of variation (CV) (the log-scale variance equals $\ln[1 + \mathrm{CV}^2]$) were selected based on the following observations. The posterior distributions of the PBK model parameters (Yang et al., 2012) were sampled 500 times each for five exposure concentrations ranging from 0.016 to 160 ppm (by factors of 10)[3] and the associated PBK metric values (for the occupational exposure scenario) were computed for each sampling. As discussed elsewhere, the human ppm-to-PBK metric conversion is linear (for this range of ppm exposure levels); the factor of 0.00373 was associated with the average of the 2500 generated PBK metric values. Similarly, a CV of 0.74 was consistent with the variation observed across all those generated PBK metric values (conditional on the value of the mean).

The cut points on cumulative PBK metric values used to assign person years of observation to four exposure groups were those shown in Table 2 (second column) multiplied by 0.00352 (the conversion factor obtained when using PBK model parameter values equal to the means of each posterior distribution).

For each of the 3000 simulated data sets, the unconstrained and constrained maximization of the log-likelihoods was completed just as described in Section 2.5 above. For interpretation of the results of the uncertainty analysis the Bayesian Information Criteria (BICs) were used to evaluate the strength of the evidence for or against any given model. The BIC is defined as,

$$\mathrm{BIC} = -2 * \mathrm{MLL} + \ln(n) * \mathrm{parms}, \tag{12}$$

where MLL, is the maximized log-likelihood, n is the number of observations, and parms is the number of parameters in the model. For the joint log-likelihoods (across mouse and human data sets) that we are analyzing here, $n = 8$ (four dose groups each for the mice and humans); $parms = 5$ for the unconstrained model (mouse and human data fit separately and independently) and $parms = 4$ for the constrained model (see Eq. (7) and associated text for a discussion of the reduction in the number of parameters under the constraint of equal risk at the DOI).

Lower values of the BIC indicate a better model. The BIC (like other information criteria) "rewards" a model for better fit (greater log-likelihood) but "penalizes" a model that uses more parameters to achieve a better fit. Put another way, the BIC rewards fit and parsimony.

A model comparison heuristic was introduced by Jeffreys (1961) and refined by Kass and Raftery (1995) (Table 3); it provides a categorization of the strength of the evidence for or against a given model, relative to another model. In our case, ΔBIC was defined with the unconstrained model as the referent, ΔBIC = BIC

[3] These exposure levels were those reported in Esmen et al. (2007a,b) as the nominal chloroprene levels for their exposure classes (see their Table 2).

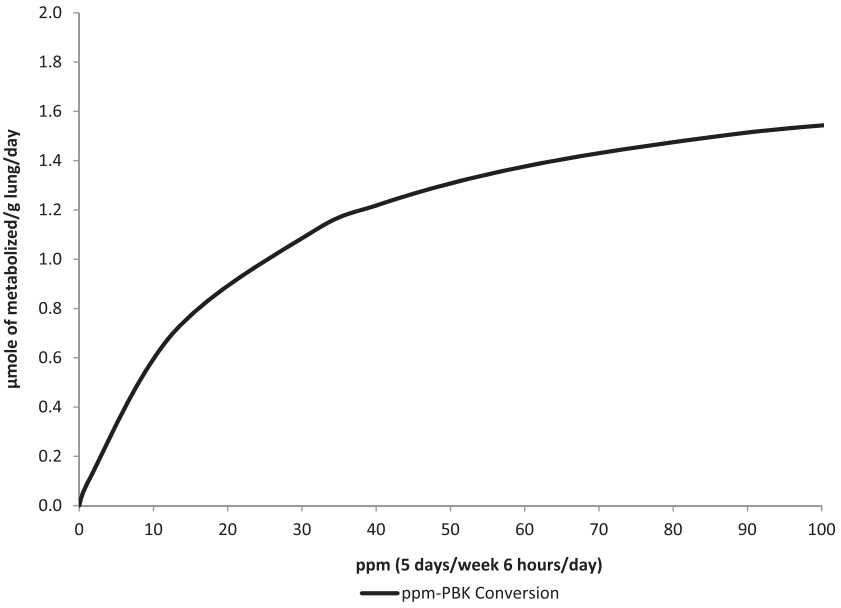

Fig. 2. Relationship between experimental exposure levels and PBK metric values; female mice.

(constrained) − BIC (unconstrained). Therefore, negative values of the ΔBIC favor the constrained model; positive values favor the unconstrained model. The results of the uncertainty analysis were summarized by tabulating the number of iterations of the simulations for which the constrained model falls in each of the evidence categories.

3. Results

The animal data set (Table 1) was not well described by the multistage model, when the doses were expressed in terms of the ppm exposure levels. The *p*-value for goodness-of-fit was 0.0046, a *p*-value indicating inadequate fit of the model to the data (*p*-values of greater than 0.10 are considered an adequate fit (USEPA, 2005)). The use of the PBK dose metric resulted in an adequate fit of the multistage model to the animal data (*p*-value = 0.44). Because of the saturation of metabolism in the lungs of female mice within the range of the experimental exposures (Fig. 2), the use of the internal PBK dose metric better correlated with the lung tumor incidence in the mouse than the external ppm dose metric. The PBK transformation was successful with respect to making differences in delivered dose accord with differences in response rates, when a multistage model represents the underlying carcinogenic process for the selected respiratory system cancer response.

The unconstrained, maximized log-likelihoods for the animal models were AMLLs = −105.758 (for the standard, ppm metric) and AMLLp = −101.049 (when using the PBK metric). The increase in the log-likelihood with use of the PBK metric is also indicative of a better fit, relative to use of the ppm exposure levels.

The human dose–response data (Table 2), were best fit by a relative risk model (Eq. (3)) with a slope (β) of zero and $\alpha = 0.74$. The fact that $\beta = 0$ is consistent with the absence of a dose–response relationship between cumulative exposure and respiratory system cancer deaths in those workers.[4] This was true whether or not the dose was expressed in terms of ppm-years or (µmole/g lung/day)-years, at least partially because the PBK transformation in humans was linear for the relatively low exposure levels experienced by this cohort (Fig. 3). The maximized log-likelihood for the relative risk model with 0 slope was HMLLs = HMLLp = 849.396 (regardless of the dose metric used).

Therefore, the "base case," unconstrained maximized combined log-likelihoods were,

$$\begin{aligned} \text{UMLLs} &= 743.638 \\ \text{UMLLp} &= 748.347 \end{aligned} \tag{13}$$

for the ppm exposure metric and for the PBK metric, respectively (Table 4).

3.1. Human–animal comparison of chloroprene risk estimates

The constrained optimization considered the animal and human data simultaneously, and maximized the *sum* of the animal and human log-likelihoods subject to one constraint, that the extra risk for the two fitted models be the same at the DOI. For the ppm exposure metric, the maximum constrained log-likelihood was attained when the relative risk slope was $\beta = 0.0017$ (per ppm-year). For that slope estimate, HMLLs(β) = 848.345, AMLLs(β) = −118.063 and therefore CMLLs = 730.282 (Table 4). The comparison of the constrained maximum log-likelihood to the unconstrained maximum log-likelihood (UMLLs = 743.638) indicates a statistically significant difference (*p*-value = 2×10^{-7}). This indicates that the animal- and human-based risks at 1 ppm are not the same (i.e., rejection of the null hypothesis). For the PBK metric, the DOI was set to 0.00352 µmole of CD metabolized/g lung/day, the PBK dose-metric that corresponds to an occupational exposure of 1 ppm. Under the constraint that the animal extra risk was the same as the human extra risk at that dose, the maximum constrained log-likelihood was attained when the relative risk slope was $\beta = 0.125$ (per (µmole/g lung/day) – years), and HMLLp(β) = 848.676, AMLLp(β) = −101.254, and therefore CMLLp = 747.422.

The PBK metric provides consistent cross-species low-dose risk estimates (the *p*-value for the test of the null hypothesis equals 0.17). The null hypothesis of equal risk at the PBK dose of 0.00352 µmole/g lung/day would not be rejected at the typical

[4] For the relative risk model, the slope was constrained to be non-negative. No evaluation was conducted to determine if negative values for the slope were better than zero. It was considered implausible that chloroprene exposure would *reduce* respiratory cancer risk.

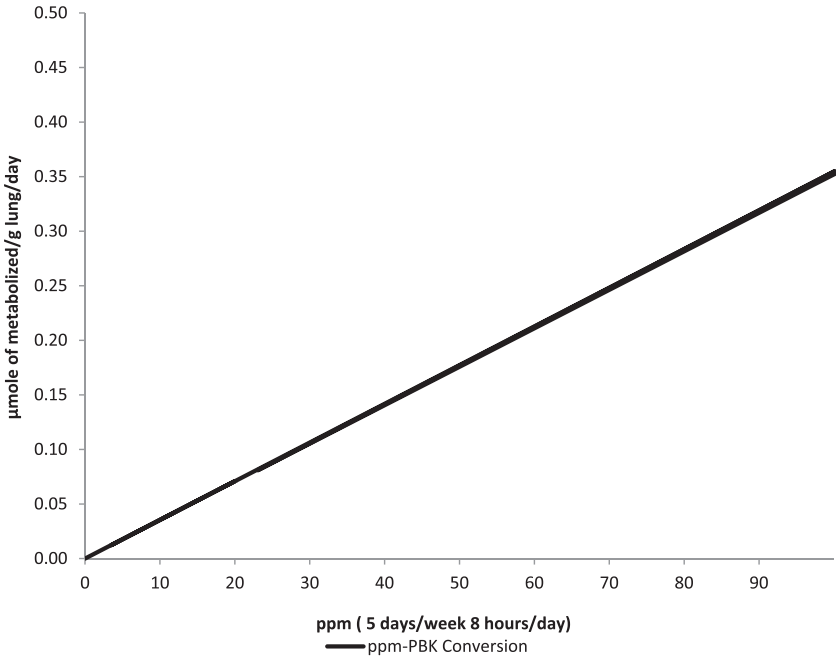

Fig. 3. Relationship between occupational exposure levels and PBK metric values; humans.

0.05 level of significance. Not only did the PBK transformation of doses result in a substantially improved model fit to the animal data, it also reconciled cross-species predictions of risk estimates for low doses.

Table 4
Unconstrained and constrained maximized log-likelihoods.

Dose-metric	Animal	Human	Combined
Unconstrained			
ppm metric	−105.758	849.396	743.638
PBK metric	−101.049	849.396	748.347
Constrained			
ppm metric	−118.063	848.345	730.282
PBK metric	−101.254	848.676	747.422

Naturally, the unconstrained fit to the animal data provided the best fit. Although the constrained fit to the animal data (where the animal risk at the DOI was constrained to equal the human risk at the DOI) was not as good as the unconstrained fit, the predicted probabilities of response were still well within the (1 SE) error bars associated with the observed response rates (Fig. 4). Importantly, the constrained curve had a less steep slope at low doses, which conforms better to the (at most) shallow slope for the human dose–response. The achievement of a shallow low-dose slope with enough curvature to match the observations at the higher

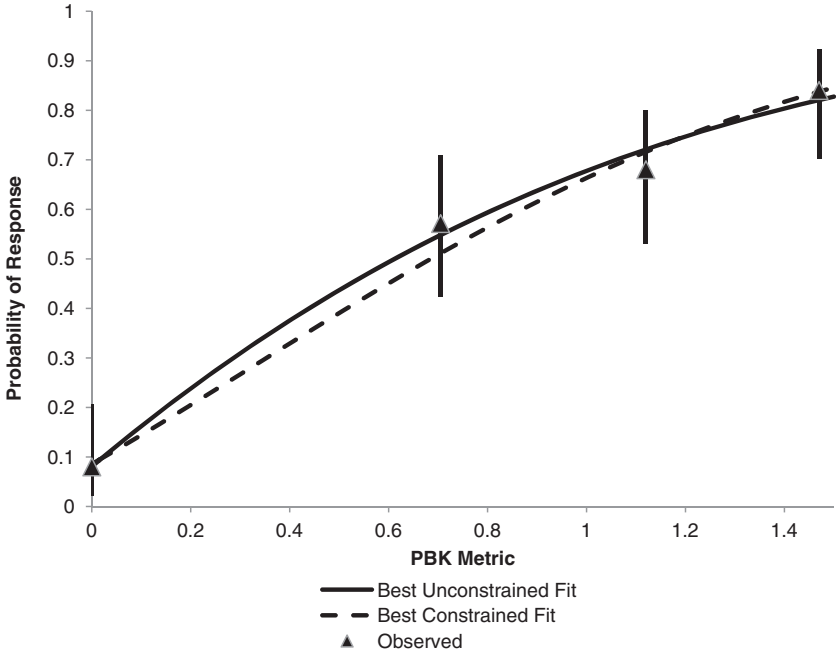

Fig. 4. Comparison of best unconstrained and constrained fits to animal data.

Table 5
Evidence for and against the constrained model, by exposure metric.[b]

ΔBIC[a]	Strength of evidence	No. simulated cohort data sets in each category	
		ppm metric	PBK metric
<−10	Very strong evidence *for* constrained model	0	736
−10 to −6	Strong evidence *for* constrained model	1	284
−6 to −2	Positive evidence *for* constrained model	16	236
−2 to 2	Not much evidence either way	46	162
2 to 6	Positive evidence *against* constrained model	131	63
6 to 10	Strong evidence *against* constrained model	259	13
>10	Very strong evidence *against* constrained model	1047	6

[a] ΔBIC = BIC(constrained) − BIC(unconstrained).
[b] Each simulated cohort data set was subject to constrained and unconstrained maximum likelihood estimation. The final two columns shows the number (out of 1500) of those data sets that had different degrees of support for or against the constrained model, depending on the choice of exposure metric.

experimental exposure levels is what allows for a consistent risk estimate at the DOI.

3.2. Uncertainty analyses

Uncertainty in estimated human exposures had an interesting effect on the comparison of the constrained and unconstrained models (Table 5). For the models applied to the ppm metric, exposure uncertainty implied a range of estimates that predominantly did not support the constrained model; all but 63 (of 1500) simulated exposure runs demonstrated evidence *against* the constrained model and, therefore, against the hypothesis that mice and humans have equal risk at 1 ppm (when risks were equilibrated on the basis of ppm exposure levels). When the PBK metric was used, there was a notable shift to values that favor the constrained model. A total of 1256 runs demonstrated evidence *for* the constrained model (nearly half were consistent with very strong evidence in favor of the constrained model and, therefore, for the equality of animal and human risks at low doses). The ability to eliminate one parameter in the optimization was of key importance, especially when the log-likelihoods for the constrained and the unconstrained models were similar. The ΔBIC for the base case (no uncertainty) constrained model using the PBK metric was −0.23, i.e., little or no evidence for or against it relative to the unconstrained model. This result is consistent with the failure to reject the null hypothesis of no difference in risk across species at the PBK dose of interest.

4. Discussion and conclusions

The analysis described here presents a new method to compare and test risk predictions across species for lifetime extra cancer risk. It requires that specific methods be applied as appropriate to the type of data available, but all having the goal of predicting lifetime extra cancer risk. Thus, for the epidemiological data, relative risk Poisson modeling linked to life-table calculations yields the necessary risk estimates. For the animal bioassay data, multistage modeling is applied. Those two sides of the analysis were subject to a formal statistical evaluation that addressed hypotheses of interest using likelihood procedures.

This approach allows for reproducible and consistent comparisons of experimental and/or observational data that are commonly used for risk assessment purposes. In the specific case of CD, the results of applying this approach indicate that external, concentration-based estimates of exposure to CD are not the appropriate dose metric for estimating comparable risk estimates across species. Even when accounting for one of the largest uncertainties associated with the use of epidemiological data for dose–response assessment, i.e., reconstructing occupational human exposure levels, there was little or no statistical support for the hypothesis that human and animal low-dose risks are equivalent when exposure was expressed in terms of ppm air concentration. Conversely, the use of the PBK metric, daily amount of CD metabolized at the target per gram of tissue, in the dose–response models provided better fit of the models to the data due to the ability of the PBK metric to account for the cross-species metabolic differences. It also resulted in comparable risk estimates across species at the dose of interest, and more generally, at all doses less than or equal to the dose of interest.

The evaluation of the animal and human data using the PBK metric provided cancer slope factors between 2.9×10^{-5} and 1.4×10^{-2} per ppm, with the maximum-likelihood estimate of 6.7×10^{-3} per ppm. The human equivalent cancer slope factor estimated based on the incidence of lung tumors in female mice (the most sensitive sex and species) reported in the EPA Toxicological Review (2010) is 6.5×10^{-1} per ppm (adjusted for exposure 6/24 h and 5/7 days). This slope factor is approximately 100 times greater than the maximum-likelihood estimate determined with the current approach.

While the current adjustment for pharmacokinetic differences across species results in comparable risk estimates, there are

Table 6
Evaluation of the presence of pharmacodynamic differences across species.

Relative pharmacodynamic sensitivity	Mouse PBK metric value (μmole of CD metabolized/g lung/day)	Mouse metric/human metric	Test of equality of risks at the specified PBK doses (*p*-value)[a]
Humans more sensitive	0.0845	24	0.001
	0.0282	8	0.029
	0.00845	2.4	0.056
Humans equally sensitive	0.00352	1	0.17
Humans less sensitive	0.00282	0.8	0.22
	0.000845	0.24	0.54

[a] *P*-values are from the test of various null hypotheses, i.e., that the risk at the specified mouse metric values is equal to the risk at the human PBK metric value of 0.00352 μmole/g lung/day (the constrained maximum likelihood calculations). The alternative hypotheses are that there is no such constraint; the mouse and human models are independent so do not necessarily predict equivalent risks at the specified doses.

additional factors that could be considered to further refine the evaluation. These could include species-specific differences in detoxification and pharmacodynamics.

In the case of CD, the data are not currently available to estimate or model the magnitude of species differences in such additional factors. However, the current analysis approach provides evidence that, if and when such data become available they will demonstrate that humans are equally or less sensitive, but not more sensitive than mice, at the low levels of CD exposure investigated. That "working hypothesis" results from the analysis results shown in Table 6. If one assumes that risk is equal when the human PBK metric value is 0.00352 μmole CD metabolized/g-lung/day and the mouse metric value is at different levels (greater or less than 0.00352), equivalence of risk was only supported (having p-values greater than 0.05) when the proposed equivalent-risk mouse dose was less than or equal to about 2.4 times the human dose of 0.00352. The working hypothesis of lower human low-dose risk still remains to be tested formally with data specifically obtained and appropriate for that purpose. Until then, the results of the current analyses suggest that humans are equally or less sensitive than mice to equivalent low-dose CD exposures.

Conflict of interest

B.C. Allen: Sub-contract to ENVIRON International Corporation and The Hamner Institutes for Health Sciences. C. Van Landingham: Contract to ENVIRON International Corporation. Y. Yang: Personal fees from International Institute of Synthetic Rubber Produces, Inc. (IISRP). A.O. Youk: Grants from International Institute of Synthetic Rubber Produces, Inc. (IISRP) and personal fees from DuPont Chemical Company. G.M. Marsh: Grants from International Institute of Synthetic Rubber Produces, Inc. (IISRP), personal fees from DuPont Chemical Company. N. Esmen: Nothing to disclose. P.R. Gentry: Contract to ENVIRON International Corporation. H.J. Clewell III: Personal fees from International Institute of Synthetic Rubber Produces, Inc. (IISRP). M.W. Himmelstein: Nothing to disclose.

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Appendix A. Formulae for calculating extra risk using a life-table method

The probability of disease occurrence (incidence or mortality) between ages x_1 and x_2 may be expressed as:

$$p(0) = \int_{x_1}^{x_2} h(x)S(x)dx \tag{A1}$$

where $S(x)$ is the probability of survival to age x given survival to age x_1 and $h(x)$ is the instantaneous hazard of disease occurrence at age x. This integral can be approximated by a sum:

$$p(0) = \sum_{i=1}^{n} p(i)S(i) \tag{A2}$$

where the age interval $[x_1, x_2]$ has been divided into n subintervals with the ith subinterval having width $\Delta(i)$, $i = 1,\ldots, n$, $p(i)$, representing the probability of disease occurrence in the ith age interval, is calculated as:

$$p(i) = q_c(i)\Delta(i), \tag{A3}$$

and $S(i)$, representing the probability of surviving to the beginning of the ith age interval given survival to age x_1, is calculated as $S(1) = 1$ and:

$$S(i) = \prod_{j=1}^{i-1} \exp[-q_a(j)\Delta(j)] = \exp\left[-\sum_{j=1}^{i-1} q_a(j)\Delta(j)\right], \quad i > 1 \tag{A4}$$

where $q_c(i)$ and $q_a(i)$ are the cause-specific rate of occurrence and all-cause death rates for the ith age interval obtained from standard rate tables. An alternative to (Eq. (A4)) is given by:

$$S(i) = \prod_{j=1}^{i-1} [1 - q_a(j)\Delta(j)], \quad i > 1, \tag{A5}$$

which encompasses slightly different interpretations of the standard rates. These 2 expressions generally agree closely.

If the subintervals correspond to individual years, (Eqs. (A2) and (A4)) take on the simplified forms:

$$p(0) = \sum_{i=x_1}^{x_2} q_c(i)S(i), \tag{A6}$$

and:

$$S(i) = \prod_{j=x_1}^{i-1} \exp[-q_a(j)] = \exp[-\sum_{j=x_1}^{i-1} q_a(j)] \tag{A7}$$

Once the background rates q_c and q_a are selected, these equations completely determine $p(0)$. These same formulae are used to calculate the probability of response, $p(D)$, from a particular exposure pattern, D, by replacing the rates q_c and q_a by the appropriate modification that accounts for the model-predicted effect of exposure on these rates. The appropriate modifications depend upon the form of the dose–response model estimated from the epidemiologic data, and the assumed exposure pattern. If the dose–response model predicts relative risk as a function of some exposure metric, then:

$$q_c(i) \text{ is replaced by } q_c(i)R(i) \tag{A8}$$

and:

$$\begin{aligned} &q_a(i) \text{ is replaced by} \\ &q_a(i) - q_c(i) + R(i)q_c(i) = q_a(i) + q_c(i)[R(i) - 1], \end{aligned} \tag{A9}$$

where $R(i)$ is the relative risk predicted by the dose-response model, i.e., $R(i) = 1 + \beta * D(i)$, where $D(i)$ is the cumulative dose at age i from exposure pattern D. The latter replacement involves subtracting from the total death rate the background death rate from the disease of interest, and adding back this contribution adjusted by the effect of exposure.

Once $p(0)$ and $p(D)$ have been calculated, the extra risk from exposure pattern D is computed as:

$$[p(D) - p(0)]/[1 - p(0)] \tag{A10}$$

This extra risk is what will be compared with the animal-based extra risk estimate.

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.yrtph.2014.07.001.

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